Megazyme

www.megazyme.com

PROTEIN DIGESTIBILITY

ASSAY PROCEDURE

Animal-Safe
Accurate Protein Quality Score
(ASAP-Quality Score Method)

for determination of the

Protein Digestibility Amino Acid Score
(PDCAAS)

K-PDCAAS 06/18

(50 Assays per Kit)

Manufactured under license with Medallion Labs,
U.S. Pat. No. 9,738,920 and additional patents pending

Medallion Labs

INTRODUCTION:

Protein is an important nutritional component for all mammals. Unlike most plants and microorganisms that can biosynthesise all twenty standard amino acids needed for health and reproduction, typical mammals cannot synthesise all amino acids for survival. These amino acids which cannot be synthesised are referred to as the essential amino acids and are required to be obtained through the diet by ingesting protein-containing foods comprised of these essential amino acids. Digestive enzymes can hydrolyse peptide bonds in the ingested proteins to release individual amino acids and small peptides which can be absorbed into the body, providing nutritional benefits.

International and local governmental regulatory bodies throughout the world have introduced standards by which the quality of protein in a food product may be judged for its completeness in delivery of the essential amino acids needed for human growth and sustenance. These regulatory requirements not only assess the compositional presence of the essential amino acids but the digestibility of the proteins and relative efficiency of the release of the amino acids present. The quality of a protein is judged from a regulatory standpoint as a function of the efficiency of protein digestion multiplied by the presence of essential amino acids. This is in essence the foundation of the most widely accepted protein quality test, known as the Protein Digestibility Corrected Amino Acid Score (PDCAAS) test, required by most regulatory bodies when asserting a protein content claim on a commercial food product.

Traditionally, protein digestibility has been evaluated *in vivo* using rat subjects to measure the amount of protein digestion that occurs when fed a protein containing product. While yielding suitable results this *in vivo* measurement process is costly and time consuming.

Additionally, this type of testing is prohibitive to food companies and individuals which uphold animal testing bans for compassionate reasons.

The Animal-Safe Accurate Protein Quality Score (ASAP-Quality Score) provided by this kit (**K-PDCAAS**), is an *in vitro* enzyme digestion method that has a very high correlation to the rat digestion model and uses the same casein standard as a completely digestible control.

NOTE: For this method each sample must also be analysed for amino acid profile, including cysteine and methionine as well as tryptophan. Analysis of protein by Dumas and moisture are required for reporting purposes.

PRINCIPLE:

Protein samples are digested by pepsin in dilute HCl (pH 2) followed by digestion with trypsin and chymotrypsin in a neutral buffer to simulate the physiological conditions of gastric and intestinal digestion, respectively (1, 2).

(1) Proteins $\xrightarrow{\text{(pepsin; pH 2.0, 37°C)}}$ proteins + peptides + amino acids

(2) Proteins $\xrightarrow{\text{(trypsin + chymotrypsin; pH 7.4, 37°C)}}$ proteins + peptides + amino acids

Undigested proteins are removed by precipitation with trichloroacetic acid. Amine groups of amino acids made available for reaction by the digestion are quantified by the reaction with ninhydrin to form ruhemann's purple (3).

(3) Ninhydrin + α-amino acids $\xrightarrow{\text{(pH 5.5, 70°C)}}$ Ruhemann's purple + RCHO + H_2O + CO_2

The amount of ruhemann's purple formed in this reaction is proportional to the amount of reactive α-amino acids present in the sample and is measured by the increase in absorbance at 570 nm. When corrected for the relative reactivity of certain α-amino acids, an *in vitro* digestibility score can be calculated. This digestibility score, in conjunction with the essential amino acid analysis of the sample, is used to calculate the PDCAAS result.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The assay is specific for the measurement of α-amino acids in all types of food products and protein concentrates.
The range of this method is from 0 to 1 for *in vitro* digestibility. The range of this method is from 0 to 1 for PDCAAS.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.
For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme website.

KITS:

Kits suitable for performing 50 assays are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Pepsin (125 mg)
Stable for > 2 years below -10°C.

Bottle 2: Trypsin (250 mg)

Stable for > 2 years below -10°C.

Bottle 3: Chymotrypsin (125 mg)
Stable for > 2 years below -10°C.

Bottle 4: L-Glycine (500 mg)
Stable for > 5 years at room temperature.

Bottle 5: **Control A** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 6: **Control B** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 7: **Control C** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 8: **Control D** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 9: **Control E** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 10: **Control F** - control powder (~ 5 g)
Stable for > 5 years below -10°C.

Bottle 11: Casein control powder (~ 5 g)
Stable for > 5 years at room temperature.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Pepsin Solution (1 mg/mL) – Weigh 1 mg of Pepsin (bottle 1) per sample into a suitably sized centrifuge tube. Add 2 additional mg so that there is extra for pipetting. Add 1 mL of the 0.06 N HCl per mg of Pepsin to the centrifuge tube. Lightly vortex to mix. Example: 15 samples in batch = (17 x 1 mg) 17 mg. Add 17 mL (17 x 1 mL) of 0.06 N HCl.
Note: Make fresh daily, use within 30 min.

2 & 3. Trypsin/Chymotrypsin Solution (5 mg/mL) – Weigh 1 mg of Trypsin (bottle 2) and 1 mg of Chymotrypsin (bottle 3) per sample into a suitably sized centrifuge tube. Add 2 additional mg of each enzyme so that there is extra for pipetting. Add 200 µL of the 0.001 N HCl per sample (plus an additional 400 µL) to the centrifuge tube. Lightly vortex to mix. Example: 15 samples in batch = (17 x 1 mg) 17 mg of Trypsin and Chymotrypsin. Add 3400 µL (17 x 200 µL) of 0.001 N HCl.
Note: Make fresh daily, use within 30 min.

4. To prepare the 10 mM L-glycine stock, weigh 37.5 mg of L-glycine into a 50 mL volumetric flask. Add approx. 25 mL sodium acetate buffer (50 mM, pH 5.5) and stir until dissolved. Bring to volume (50 mL) with sodium acetate buffer (50 mM, pH 5.5), cap and invert several times to mix. Transfer to a sealed container. Use to prepare the L-glycine standards (Table 1, page 7). Stable for up to three months at 4°C.

5-11. Use the contents of bottles 6-11 as supplied. Stable for > 5 years below -10°C (bottles 5-10) or at room temperature (bottle 11).

PREPARATION OF REAGENT SOLUTIONS (NOT SUPPLIED):

1. Ninhydrin Reagent (2% solution)

(Sigma cat. no. N7285 or equivalent). Use as supplied.

NOTE: After using ninhydrin, purge the stock bottle with nitrogen and seal with parafilm before returning to the refrigerator. If ninhydrin is not capped with nitrogen, oxidation will occur and the reagent will no longer work sufficiently.

2. Hydrochloric acid (0.06 N, pH 2.0): 1 L

Place approx. 900 mL of distilled water in a 1 L beaker. Add 5 mL of 12 N HCl while stirring. Adjust the pH to 2.0 with 2 N NaOH. Transfer to a 1 L volumetric flask and bring to volume (1 L) with distilled water. Transfer to a suitable sealed container. Store for up to 1 year at room temperature.

3. Trichloroacetic acid (40% w/v): 100 mL

Add 40 g of trichloroacetic acid (Sigma cat. no. T6399) to approx. 80 mL of distilled water and dissolve by stirring. Make to volume (100 mL) with distilled water. Store for up to 1 year at room temperature.

4. Hydrochloric acid (0.001 N, pH 3.0): 500 mL

Place approx. 450 mL of distilled water in a 500 mL beaker. Add 5 mL of 0.1 N HCl while stirring. Adjust the pH to 3.0 with 0.1 N HCl/NaOH. Transfer to a 500 mL volumetric flask and bring to volume (500 mL) with distilled water. Transfer to a suitable sealed container. Store for up to 1 year at room temperature.

5. Tris Buffer (1.0 M, pH 7.4): 250 mL

Place 150 mL of distilled water in a beaker. Add 30.29 g of Tris base while stirring. Slowly add 15 mL 12 N HCl. Adjust the pH to 7.4 with 1 N HCl and transfer to a 250 mL volumetric flask. Bring to volume (250 mL) with distilled water and mix. Sterile filter buffer and transfer to a sealed container. Store for up to 4 months at room temperature.

6. Sodium acetate buffer (50 mM, pH 5.5): 1 L

Add 2.05 g of anhydrous sodium acetate to approx. 850 mL of distilled water. Once mixed, carefully add 1.58 mL of glacial acetic acid. Adjust the pH to 5.5 by the addition of 2 N sodium hydroxide solution. Transfer to a 1 L volumetric flask and make

to volume with distilled water. Store for up 6 months at room temperature.

7. Reagent Alcohol (50% v/v): 1 L

Add reagent alcohol (VWR Scientific Products cat. no. BDH-1156-4LP) to an equal volume of distilled water and mix thoroughly. Store for up 1 year at room temperature.

EQUIPMENT (RECOMMENDED):

1. 96 well microplate (e.g. clear flat-bottomed, glass or plastic).
2. Centrifuge Tubes (50 mL, 15 mL and 2 mL), e.g. cat. no. 21008-240, cat. no. 89039-670 and cat. no. 22179-008 (VWR Scientific Products).
3. Centrifuge Tubes 50 mL, round bottom, e.g. cat. no. P20504MPI (Beckman Coulter).
4. Disposable funnels, e.g. cat. no. 30246-021 (VWR Scientific Products).
5. Culture Tubes, 16 x 100 mm and 13 x 100 mm, e.g. cat. no. 47729-576 and cat. no. 47729-572 (VWR Scientific Products).
6. Culture Tube Caps, 16 mm and 13 mm, e.g. cat. no. 60828-766 and cat. no. 60828-746 (VWR Scientific Products).
7. Media bottles 1 L, 500 mL and 250 mL, e.g. cat. no. 16157-191, cat. no. 16157-169 and cat. no. 6157-136 (VWR Scientific Products).
8. Water bottles, e.g. cat. no. 10111-950 (VWR Scientific Products).
9. Disposable Pipets, e.g. cat. no. 414004-001 (VWR Scientific Products).
10. Micro-pipettors, e.g. Gilson Pipetman® (20 µL, 200 µL and 1 mL).
11. Positive displacement pipettor, e.g. Eppendorf Multipette® with 5.0 mL and 50 mL Combitip®.
12. Stop clock.
13. pH Meter.
14. Analytical balance (capable of weighing to +/- 0.0001 g).
15. Microplate reader set at 570 nm.
16. Heated water bath (capable of 95°C).
17. Shaking incubator (capable of 37°C and 70°C).
18. Centrifuge with rotor capable of 15,000 x *g*, e.g. Avanti J-26XPI cat. no. J326XPI-IM-4AB (Beckman Coulter).
19. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).

STANDARD ASSAY PROCEDURE:

NOTES:

1. Calibration standards are only needed if generating a calibration curve for calculating *in vitro* protein digestibility. A previously generated curve can be used but only if a casein control sample is tested as a cross reference to the curve.
2. For each batch of samples that is applied to the PDCAAS procedure a blank sample (i.e. an empty tube to which all reagents are added) must also be included.

A. SAMPLE EXTRACTION:

1. Mill cereal, plant or food product to pass a 0.5 mm screen.
2. Accurately weigh approx. 500 mg of milled, calibration or casein control sample into a 50 mL Beckman centrifuge tube. Tap the tube to ensure that all of the sample drops to the bottom of the tube.

3. Gastric and Intestinal Digestion:

   a. Add 19 mL of HCl (0.06 N) and cap the tube. Mix thoroughly by vortex and incubate for 30 min at 37°C in a hot air, shaking incubator set at 300 rpm.
   b. Add 1 mL of pepsin solution (bottle 1) to each sample and cap each tube. Mix thoroughly by vortex and incubate for 60 min at 37°C in a hot air shaking incubator set at 300 rpm.
   c. After the pepsin incubation is complete, remove samples and adjust the pH to 7.4 by the addition of 2 mL of 1.0 M Tris buffer, pH 7.4. Cap the tubes and mix each sample thoroughly by vortex.
   d. Add 200 µL of Trypsin/Chymotrypsin mixture to each sample, mix thoroughly by vortex and incubate for 4 h at 37°C in a hot air, shaking incubator set at 300 rpm.
   e. At the end of the Trypsin/Chymotrypsin incubation place the samples in a boiling water bath for 10 min.
   f. Remove all samples from the boiling water bath and mix thoroughly by vortex.

4. Allow the samples to cool to room temperature for at least 20 min and transfer 4 mL of each to a 16 x 100 culture tube. **Note: Remaining sample can be frozen for future analysis. Store at -80°C.**
5. Add 1 mL of 40% TCA solution, cap and mix thoroughly by vortex. Incubate the samples at 4°C overnight (at least 16 h). **Note: Analysis can be held at this point.**
6. Transfer approx. 1.75 mL of the sample (avoiding the precipitate) to a 2 mL centrifuge tube and centrifuge for 10 min at 15,000 x *g*

at room temperature.

7. Make a 10-fold and 20-fold dilution in acetate buffer (50 mM, pH 5.5) into a culture tube for all samples. The blank and controls (A-F) only require a 10-fold dilution, while the casein requires a 20-fold dilution.
   Note: All samples require a minimum of a 10-fold dilution to bring the TCA concentration down to a level that will not affect the Colourimetric Determination of Amines (Section C).
8. All diluted supernatants of the sample solutions, including the sample blanks, calibration samples and the casein control samples, are applied to the Colourimetric Determination of Amines (see section C).

B. PREPARATION OF THE L-GLYCINE CALIBRATION STANDARDS:

Prepare the standard L-glycine standard solutions in 15 mL culture tubes as described in the **Table 1** and treat as samples in the Colourimetric Determination of Amines (see section C).
Stable for up to 1 month at 4°C.

Table 1. Preparation of the L-gLycine calibration standards.

L-Glycine Standards (mM)		Sodium Acetate (50 mM, pH 5.5)	L-Glycine standard
ST 11	1	9 mL	1 mL of 10 mM L-glycine
ST 10	0.75	9.25 mL	0.75 mL of 10 mM L-glycine
ST 9	0.5	9.5 mL	0.5 mL of 10 mM L-glycine
ST 8	0.25	9.75 mL	0.25 mL of 10 mM L-glycine
ST 7	0.1	9.9 mL	0.1 mL of 10 mM L-glycine
ST 6	0.075	9 mL	1 mL of STD 10
ST 5	0.05	9 mL	1 mL STD 9
ST 4	0.025	9 mL	1 mL STD 8
ST 3	0.01	9 mL	1 mL STD 7
ST 2	0.007	9 mL	1 mL STD 6
ST 1	0.005	9 mL	1 mL STD 5
ST 0	0	10 mL	0

C. COLOURIMETRIC DETERMINATION OF AMINES:

NOTE: For each batch of samples that is applied to the Colourimetric Determination of Amines, **a calibration curve of L-glycine must be performed concurrently using the same batch of reagents (see section B).**

1. Set up the reactions for the Colourimetric Determination of Amines as shown in **Table 2** using the suggested format in the microplate (**Table 3**).

Table 2. Reaction set up for the colourimetric determination of amines.

Wavelength:	570 nm
Cuvette:	96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature:	70°C
Final volume:	0.300 mL
Sample solution:	0-1 mM of L-glycine (in a 0.100 mL sample volume)

Pipette into wells	Sample	Standard
sample solution (incl. blank)	0.100 mL	-
standard solutions (glycine)	-	0.100 mL
ninhydrin reagent* (2%)	0.050 mL	0.050 mL
Place the lid on the plate and cover with foil and place on a pre-heated tray in a hot air incubator*** at 70°C for 35 min at 100 rpm. Remove the plate from the incubator. Keep covered with foil while allowing to cool for 10 min. **Then add:**		
reagent alcohol (50% v/v)	0.150 mL	0.150 mL
Mix** and read the absorbances of the solutions at 570 nm against the L-glycine standard (ST 0).		

* Ensure that the ninhydrin has warmed to room temperature before use.

** For example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipettor set at 50-100 µL volume).

*** Alternatively, the microplate can be incubated in a plate reader that has a heating facility. In this instance, there is no requirement to cover with foil.

NOTE: If the absorbance of the samples is above the range of the glycine standards it is necessary to further dilute the samples and perform the Colourimetric Determination of Amines again. Calculated amine levels will need to be corrected by this dilution factor.

Table 3. Plate layout for the colourimetric determination of amines reaction.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST11	ST10	ST 9	ST 8	ST 7	ST 6	ST 5	ST 4	ST 3	ST 2	ST 1	ST 0
B	ST11	ST10	ST 9	ST 8	ST 7	ST 6	ST 5	ST 4	ST 3	ST 2	ST 1	ST 0
C	Blank	A	B	C	D	E	F	Casein	S1	S2	S3	S4
D	Blank	A	B	C	D	E	F	Casein	S1	S2	S3	S4
E	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
F	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
G	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
H	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24

ST 0 - ST 11 = L-Glycine standards 0-11
A - F = Control samples A-F
S1 - S24 = Test samples 1-24

SAMPLE PREPARATION:

1. Ground, frozen samples should be stored below -10°C and thoroughly homogenised prior to weighing.
2. Refrigerated samples should be weighed cold and returned to the fridge or freezer as soon as possible.
3. Do not allow refrigerated or frozen samples to warm to room temperature before weighing. Weigh "as is" to ensure integrity of the matrix.
4. Liquid samples should be thawed under a stream of nitrogen prior to weighing.

CALCULATION:

1. L-Glycine standard curve

Using the absorbance values generated for the L-glycine standards (0-1 mM) plot a linear standard curve of absorbance (y-axis) versus concentration (mM) (x-axis). Include the zero point (ST 0) in the curve. See **Figure 1** (page 13) as an example.
Determine the primary amine concentration (C1) of unknown samples using the following equation:

primary amine concentration (mM): Y=A*C1+B

where:
C1 = unknown concentration of primary amines (mM)
Y = absorbance
B = y-intercept
A = slope of the line

Example curve: Y = 4.2649 x C1 – 0.0105

2. Primary amine concentration corrected for dilution and weight

Calculate the primary amine concentration in the original sample solution (C2) by multiplying the value obtained above by the dilution factor and also adjusting for any deviation from nominal sample size.

C2 = C1*D*1.25*(0.5)/W

where:

C1 = concentration of primary amines in the diluted sample
D = dilution factor of the sample prior to amine determination
1.25 = dilution with TCA (all samples equal)
W = sample weight (g)
0.5 = nominal sample size (g)

3. Primary amine concentration corrected for amino acids present

Using constants for various amino acids calculate a corrected primary amine concentration (CN) for amino acids present:

CN = C2+((Prol*2*10)/(Lys*0.5*10))+(Hist*0.2*10)+(Arg*0.2*10)

where:

C2 = corrected primary amine concentration (mM)
Prol, Lys, Hist and Arg = concentration of L-proline, L-lysine, L-histidine and L-arginine respectively in the original sample
2, 0.5, 0.2 and 0.2 = constants for the various amino acids

4. Data fit comparison to literature values

Using the literature values for the rat model in **Table 4** below, fit the corrected primary amine concentration (CN) for the standard samples to the corresponding literature values using a linear regression.

Table 4. Literature values of primary amine concentrations.

Matrix	Literature Value (mM)
Control A	83
Control B	86
Control C	91
Control D	84
Control E	84
Control F	81
Casein	100

NOTE: If a previously generated correlation equation is used, adjust for the relative variability of the casein control sample.

5. *In vitro* digestibility

Using the equation from the Data Fit calculate the *in vitro* digestibility of the sample:

***In Vitro* Digestibility = (M*X+B)/100**

where:

X = corrected primary amine concentration (CN) for each sample
M = slope of the line (1.1135 in the example above)
B = y-intercept (74.125 in the example above)
100 = conversion from percentage to g

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

6. Determine the amino acid ratio and limiting amino acid

Determine the amount of amino acid for each of the samples on a g/100 g protein basis using the total Dumas protein result generated separately:

AA (g/100 g) = (AA g/100 g Sample)/(Dumas protein (%))

Calculate the ratio of amino acid (mg/g protein) in the sample with the recommended amount. **Table 5** shows the recommended amount.

Ratio = (mg/protein in sample)/(mg/g protein in reference sample)

Table 5. FAO recommended values for essential amino acids.

ESSENTIAL AMINO ACIDS for Human Nutrition	FAO Recommended Values (2011) – mg/g protein	FAO Recommended Values (1991) – mg/g protein*
Histidine	20	19
Isoleucine	32	28
Leucine	66	66
Lysine	57	58
Methionine + Cysteine	27	25
Phenlyalanine + Tyrosine	52	63
Threonine	31	34
Tryptophan	8.5	11
Valine	43	35

*1991 referenced values are required to be used for compliance with the U.S. Code of Federal Regulations.

The essential amino acid with the lowest ratio is the limiting amino acid. See **Table 6** below for example.

Table 6. Example of essential amino acid with limiting ratio.

Amino Acid	Sample Name (g/100g sample)	Sample Name (g/100 g protein)	Sample Name (mg/g protein)	1991 Reference Protein (mg/g protein)	Ratio	
L-Cysteine + L-Methionine*	1.38	2.94	29.44	25.00	1.178	
L-Tryptophan*	0.85	1.82	18.20	11.00	1.655	
L-HydroxyProline	ND					
L-Aspartic acid	3.20					
L-Threonine*	1.01	2.16	21.63	34.00	0.636	
L-Serine	1.56					
L-Glutamic Acid	6.66					
L-Proline	1.19					
L-Glycine	1.82					
L-Alanine	1.51					
L-Valine*	1.81	3.87	38.74	35.00	1.107	
L-Isoleucine*	1.45	3.09	30.94	28.00	1.105	
L-Leucine*	2.57	5.51	55.10	66.00	0.835	
L-Tyrosine + L-Phenylalanine*	3.26	6.98	69.83	63.00	1.108	
L-Lysine*	1.49	3.18	31.82	58.00	0.549	1
L-Histidine*	0.85	1.81	18.14	19.00	0.955	
L-Arginine	5.51					
Total Protein =	36.08					

*essential amino acid for nutrition

[1] limiting amino acid for sample

7. *In Vitro* PDCAAS Score

Calculate the *in vitro* PDCAAS score by multiplying the *in vitro* digestibility (Step 5) with the limiting amino acid ratio (lowest value).

PDCAAS = Digestibility x Ratio

where:

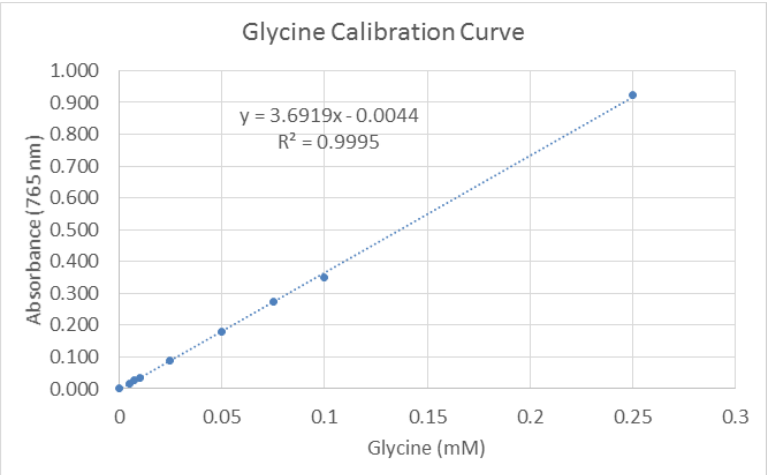
PDCAAS = *in vitro* PDCAAS score
Digestibility = *in vitro* digestibility from step 5
Ratio = ratio of the limiting essential amino acid from step 6

APPENDIX:

Linearity:

Linearity of the Colourimetric Determination of Amines reaction was determined by performing a glycine standard curve.

Figure 1. Example L-glycine calibration curve.



Accuracy:

Accuracy was determined by correlating the AA corrected ninhydrin result with that of the digestibility from literature values. **Figure 2(a-c)** displays correlation of *in vitro* ASAP-quality digestibility score (Megazyme Protein Digestibility) with literature values of rat *in vivo* digestibility score and of *in vitro* ASAP-quality PDCAAS value score with rat *in vivo* PDCAAS value. The correlation between both methods for the digestibility score and the PDCAAS value is 0.9205 and 0.9784, respectively. These values are adequate for correlations of this nature.

Figure 2(a). Table of *in vitro* ASAP-quality digestibility score (Megazyme Protein Digestibility) and literature values of rat *in vivo* digestibility score.

Matrix	ASAP-Quality Digestibility Score	Rat Digestibility Score
Casein	100.2	100.0 [1]
Rolled Oats	82.7	83.0 [1]
Lentils	85.4	86.0 [1]
Wheat	89.5	91.0 [1]
Split Pea	85.2	84.0 [1]
Sunflowers Seeds	86.0	94.0 [1]
Black-eyed peas	83.7	84.0 [1]
Kidney Beans	81.5	81.0 [1]
Peanuts Roasted High Oleic	71.0	75.0 [2]
Sunflower Kernels Roasted No Salt SL80	77.0	79.0 [2]
Peanut Butter#7	92.0	93.0 [2]
Pea Protein Bar Fruit and Nut	90.0	89.0 [2]
Oats Rolled #15	93.0	95.0 [2]
High Pro Nutty Granola Cluster	97.0	97.0 [2]
Granola Base #7 Sucrose/Canola Natural	98.0	99.0 [2]
Granola Bar #1	78.0	78.0 [2]
Granola Bar #2	84.0	84.5 [2]
Chicken Stock Concentrate Salt	86.0	85.5 [2]
Granola base #5	94.0	93.0 [2]

[1] based on published literature values

[2] based on direct analysis of the same sample by both methods

Figure 2(b). Correlation of *in vitro* ASAP-quality digestibility score with literature values of rat *in vivo* digestibility score.

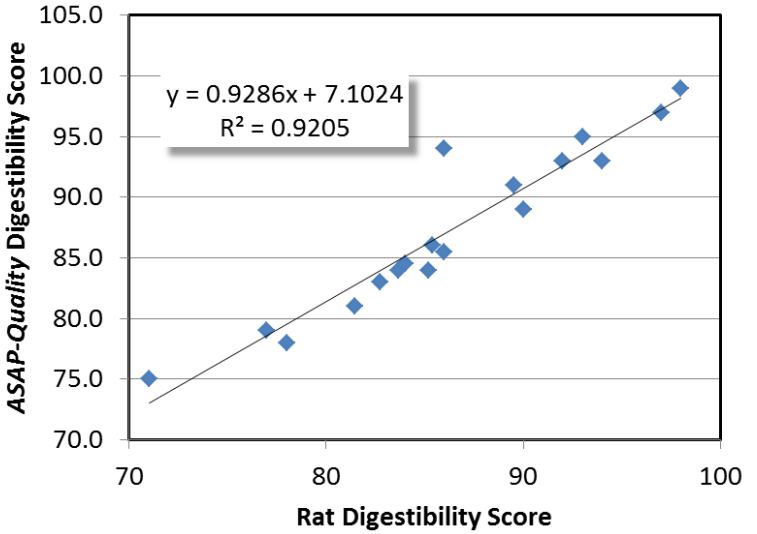
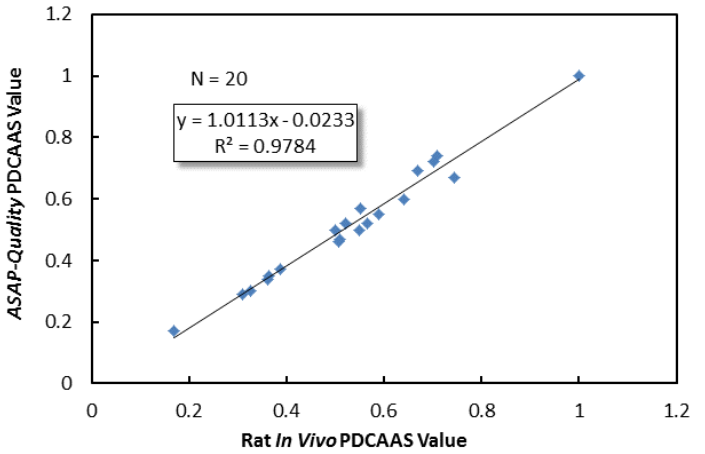


Figure 2(c). Correlation of *in vitro* ASAP-quality PDCAAS value score with rat *in vivo* PDCAAS value.



Precision:

Precision was determined by assaying 7 control samples across 5 days. The data in **Table 7** shows the glycine equivalent values obtained from those 5 batches. The pooled RSD result is below 10%.

Table 7. Glycine equivalent values obtained from 5 control samples.

Rep	Casein	Control A	Control B	Control C	Control D	Control E	Control F
Day 1	10.08	1.94	4.02	1.50	3.46	1.61	1.62
Day 2	8.47	1.59	3.45	1.31	3.23	1.43	1.43
Day 3	8.74	2.03	4.14	1.48	3.67	1.77	1.57
Day 4	8.63	1.88	3.89	1.33	3.51	1.57	1.53
Day 5	9.16	2.06	4.10	1.47	3.57	1.61	1.59
Average	9.02	1.90	3.92	1.42	3.49	1.60	1.55
SD	0.65	0.19	0.28	0.09	0.16	0.12	0.07
RSD (CV, %)	7.18%	9.87%	7.13%	6.37%	4.70%	7.59%	4.76%

Control Samples for PDCAAS Analysis:

1. Store controls in desiccator.
2. All controls will need to have an amino acid profile including cysteine and methionine as well as tryptophan performed on them.
3. Protein Dumas will also be required.
4. A moisture value should also be determined as an initial value, so that comparisons can be made should the need arise.

Ninhydrin Reagent Quality:

To retain a good quality ninhydrin reagent, it must be properly capped with nitrogen otherwise it will rapidly oxidise and the quality lost. The quality of the ninhydrin reagent can be assessed by performing a wavelength scan of the reagent solution from 300 nm to 600 nm. Good quality ninhydrin reagent will generate a high absorbance across the whole wavelength scan while low quality reagent that has oxidised will begin to completely lose absorbance at the higher wavelengths.

Use of a low quality ninhydrin reagent will result in loss of sensitivity in the Colourimetric Determination of Amines assay (**Section C**) and poor correlation values for the ninhydrin curve.

REFERENCES:

1. Plank, D. W. (2017). US Pat 9,738,920. "*In vitro* method for estimating *in vivo* protein digestibility".
2. The State of Food and Agriculture, Food and Agriculture Organization of the United Nations, 2011.

NOTES:

NOTES:

Developed by Medallion - Powered by Megazyme

Megazyme
Bray Business Park, Bray,
Co. Wicklow,
A98 YV29,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com